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Evolution of Wild-Type and Precore Mutant HBV Infection After Liver Transplantation

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Recurrence of hepatitis B virus (HBV) infection after liver transplantation is associated with varying degree of graft damage. The aim of the study was to investigate longitudinally the changes of wild-type and precore A_{1896}HBV mutant viral populations after reinfection and their impact on liver graft damage. The wild-type HBV and A_{1896}HBV strains were quantitated before and serially after orthotopic liver transplantation (OLT) in 14 hepatitis B surface antigen (HBsAg)-positive liver graft recipients (4 hepatitis B e antigen [HBeAg]+; 10 anti-HBe+). Before OLT, the wild-type precore HBV was present in all 4 HBeAg-positive patients and in 2/10 anti-HBe-positive patients; a mixed virus population was present in 6 patients; and A_{1896}HBV mutant alone in 2 patients. After OLT, A_{1896}HBV mutant appeared and gradually accumulated in 5/6 patients who had the wild-type HBV before OLT and 1 of these patients seroconverted from HBeAg to anti-HBe 52 months after transplantation. A mixed HBV population was present continuously in 6 patients before and after OLT. Of the 2 patients with A_{1896}HBV only pre-OLT, the wild type appeared in one patient and the other patient retained persistently the A_{1896}HBV mutant. There was no relationship between liver graft histology and the type of viral population at reinfection or at the end of follow up. Changes in the HBV population occur during follow up of recurrent hepatitis B in liver transplant recipients with frequent accumulation of precore A_{1896}HBV mutants, but the type of viral population does not determine the severity of hepatitis B in the graft. ***J. Med. Virol. 59:5–13, 1999.***

KEY WORDS: hepatitis B virus; precore mutant HBV; liver transplantation; HBV recurrence; hepatitis B

INTRODUCTION

Hepatitis B virus (HBV) recurrence in the liver graft, following orthotopic liver transplantation (OLT) for hepatitis B surface antigen (HBsAg)-positive cirrhosis, is associated with varying degree of graft damage—from mild chronic hepatitis to severe hepatitis with accelerated progression to cirrhosis [O'Grady et al., 1992; Samuel et al., 1993; Lucey, 1994]. In addition, acute liver failure may develop in some liver transplant recipients as a result of fulminant hepatitis (FH) or a rare syndrome, termed *fibrosing cholestatic hepatitis* (FCH) [Davies et al., 1991; Lau et al., 1992]. The introduction of continuous prophylaxis with hepatitis B immunoglobulin (HBIg) has reduced the rate of HBV recurrence and has improved 1 year survival to around 86% of patients [Devlin et al., 1994]. Nevertheless, the long-term survival in these liver transplant recipients remains lower in comparison with other OLT indications, as HBV recurrence still occurs in 30% of patients [Samuel et al., 1993].

The changes in virus population following HBV recurrence and the role of viral factors for the development of different patterns of liver graft damage are not fully understood. Longitudinal studies of HBV infection after liver transplantation provide an opportunity to understand the interactions between the wild-type and precore mutant HBV strains in a natural system, where the timing and the characteristics of virus population at the onset of infection can be defined. In an animal model using duck HBV, it was suggested that the precore mutant strain had less active replication than the wild-type duck HBV and it could be overgrown by the wild-type virus during the course of coinfection [Tong et al., 1991; Chuang et al., 1994]. In patients with chronic hepatitis B, it is believed that immune reactions in the host exert selection pressure, which is responsible for the emergence of precore mutant HBV,

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TABLE I. Patients Characteristics

	Before liver transplantation					After liver transplantation		
Pt	Age (years)	HBeAg	Anti-HBe	Anti HDV	HBV DNA (pg/ml)	HBIg	Reinfection (months)	End of follow up (months)
JC	46	+	–	–	46	+	HBsAg	56
KG	54	+	–	–	16	+	6	12
PJ	58	+	–	–	44	+	3.9	54
WM	52	+	–	–	66	+	HBsAg	38
AS	42	–	+	–	Neg	+	6.3	17
AB	37	–	+	+	Neg	–	1.7	47
GC	50	–	+	+	8	+	1.5	4+
KK	45	–	+	–	Neg	+	9.4	15
DL	42	–	+	+	Neg	+	17.2	20
JP	54	–	+	–	Neg	+	1.2	3+
FS	37	–	+	+	Neg	+	1.2	4+
RS	29	–	+	–	Neg	–	HBsAg	71
AQ	41	–	+	+	Neg	+	2.8	40
CK	56	–	+	–	9	+	1.2	21

Pt, patient; HBeAg, hepatitis B e antigen; HDV, hepatitis delta virus; HBV, hepatitis B virus; HBIg, hepatitis B immunoglobulin; HBsAg, persistent hepatitis B surface antigen positivity after orthotopic liver transplantation; + = Patient died.

and this process is facilitated by the base pairing in the stem loop encapsidation signal [Lok et al., 1994].

Since the first description of HBV mutants unable to secrete hepatitis B e antigen (HBeAg), it has been suggested that infection with these mutants is associated with FH or a more severe course of chronic hepatitis [Carman et al., 1989; Brunetto et al., 1991b, 1993a, 1993b; Liang et al., 1994]. However, HBe-minus strains of the virus are present also in patients with mild hepatitis or healthy carriers [Naoumov et al., 1992; Tur Kaspa et al., 1992] and can emerge during natural or interferon-induced seroconversion [Takeda et al., 1990]. Pretransplant infection with precore mutant HBV has been suggested as a major factor for severe HBV recurrence after transplantation [Angus et al., 1995], whereas a recent study showed that the pretransplant viral load, but not the infection with the precore mutant HBV, determines the outcome after OLT [Naumann et al., 1997]. Both studies focused only on the impact of wild-type and precore mutant HBV on graft and patient survival, but did not analyse serially the evolution of virus population following HBV recurrence.

The aim of the study was to evaluate quantitatively the changes in the wild-type and the precore A_{1896}HBV mutant viral populations after HBV recurrence in liver transplant recipients and their impact on liver graft damage.

PATIENTS AND METHODS

Fourteen patients (12 male; 2 female) who underwent OLT between 1988 and 1993 for HBV-related cirrhosis at the Institute of Liver Studies, King's College Hospital, London and developed HBV recurrence after transplantation were studied (Table I). The median age was 45.5 years (range 29–58 years). Four of these patients were from the UK, 8 from the Mediterranean region, and 2 from the Far East. Five of the patients had Delta superinfection, and all patients were negative for antibodies against hepatitis C virus (HCV) and human immunodeficiency virus (HIV). After transplantation, the patients received triple immunosuppression with prednisolone, cyclosporine, and azathioprine. Corticosteroids were usually discontinued 3–6 months post-OLT. All patients, except 2 who were transplanted before 1989, received HBIg immunoprophylaxis with 5,000 IU weekly for the first month and then 2,000 IU monthly to maintain an anti-HBs titre above 100 IU/L. Immunoprophylaxis was discontinued after diagnosis of HBV reinfection, defined as reappearance of the HBsAg in serum and in the liver graft. At the time of transplantation, 4 patients were seropositive for HBeAg and HBV-DNA. The remaining 10 patients were HBeAg negative, anti-HBe positive, with serum HBV-DNA detectable in 2 patients (8 pg/ml and 9 pg/ml, respectively). Following HBV recurrence, 3 patients developed acute liver failure (FH in 2; fibrosing cholestatic hepatitis in 1) and died within 4 months after liver transplantation. The other 11 patients developed chronic HBV infection in the graft and were followed for a median period of 38.3 months (range 12–71 months).

A total of 97 serum samples were analysed, taken before and serially after OLT with 5–10 samples per patient. Percutaneous liver biopsies were performed as part of the diagnostic evaluation with patients' informed consent when indicated clinically.

Oligonucleotide Hybridisation Assay

The pre-C/C gene of HBV was amplified from serum using a single round polymerase chain reaction (PCR), or nested PCR if needed, using synthetic primers, P1 (5′ ACGACCGACC TTGAGGCATACTTC) and P2 (5′ CTGCGAGGCGAGGGAGTTCTTCTT) for the first round or P3 (5′ GGAGGCTGTAGGCATAAATTGGTCTGCGC) and P4 (5′ GCCTGAGTGCTGTGTGGTGAGGTGAGCAA) for the nested, designed according

to the sequence of the HBV genotype D-subtype ayw 3 [Galibert et al., 1979]. The PCR mixture contained 1× PCR buffer (Gibco-BRL, Paisley, UK), 0.2 mM of each deoxynucleotide (Boehringer Mannheim, Lewes, UK), 20 pmol of each primer, 2.5 U Taq polymerase (Gibco). Ten microlitres of serum were mixed with equal volume of distillate water and microwaved for 6 min. Ten microlitres supernatant of the microwaved serum or 1 μl of the first-round product were added to the PCR mixture in each tube and overlaid with mineral oil. A step-cycle profile was programmed in a DNA Thermal Cycler 480 (Perkin-Elmer Applied Biosystem, Cheshire, UK). For the first round: denaturation at 94°C, 1 min; annealing at 56°C, 1.5 min; extension at 72°C, 3 min, for 35 cycles, with a final extension of 8 min at 72°C; for the nested: denaturation at 94°C, 1 min; annealing at 60°C, 1 min; extension at 72°C, 2 min, for 25 cycles, with a final extension of 8 min at 72°C.

An oligohybridisation assay described previously was used with several modifications [Brunetto et al., 1991a]. The detection was based on a digoxigenin detection system, instead of radioactive labelling. For blotting, nylon membranes where used instead of nitrocellulose membranes. This substitution allows the membranes to be probed several times, thus allowing standardisation of the assay and verification of the reproducibility of the results. The specificity and sensitivity of the assay was established for a range from 10 to 400 ng of amplified HBV-DNA. We used known amounts of wild-type and A_{1896}HBV mutant amplicons obtained by PCR of two plasmids -pSM2 and pSMTAG, respectively (kindly donated by Prof. H. Will, Hamburg, Germany) as a standard curve (range 10–500 ng) to allow exact measurement of the tested samples. In the preliminary experiments, we observed cross-reactivity in the oligohybridisation procedure when PCR products exceeding 500 ng were spotted on the membrane. For this reason, the semiquantitation of the PCR products before the transfer on the membrane was essential to ensure that for each sample the membrane was loaded with the amount of target DNA within the desired range. The sensitivity of detection for the minimum amount of wild-type or A_{1896}HBV in a sample with mixed HBV population was between 5% and 10% of the total viral population.

After amplification, 5 μl of the PCR products were loaded on a 1% agarose gel with known amount of same size amplicons obtained by PCR of a plasmid HBV genome. This method allowed a semiquantitative evaluation of the PCR products. Between 100 and 300 ng of the PCR product was mixed with 30μl of DNA buffer (1 M Tris buffer, 0.2% salmon sperm DNA), heat denatured (98°C for 10 min, ice-quench for 10 min), dot-blotted in duplicate through a manifold apparatus on a positively charged nylon membrane (Boehringer Mannheim), thus creating two identical membranes.

The hybridisation was performed using synthetic oligonucleotide probes specific for the wild-type sequence (TGGCTTTGGGGCATGGAC) and the precore mutant A_{1896}HBV sequence (TGGCTTTAGGGCATGGAC), labelled with digoxigenin, according to a short tail protocol (Tailing Kit, Boehringer Mannheim). The prehybridisation step was performed in 5× SSC (1× SSC: 0.15 M sodium chloride; 0.015 M sodium citrate), 0.1% *N*-lauroylsarcosine, 0.5% sodium dodecyl sulphate (SDS), 1% blocking reagent (Boehringer Mannheim), 0.1 mg/ml salmon sperm DNA at 60°C for 1 hr. The hybridisation step was carried out in a fresh prehybridisation solution plus 5 pg/ml of digoxigenin-labelled probe and the two membranes were hybridised in parallel at 60°C for 1 hr. Afterward, the membranes were rinsed twice at room temperature in 5× SSC/0.1% SDS. Stringent conditions were achieved by washing twice at 60°C for 10 min each membrane with a specific solution for each probe: for the wild-type probe: 1× SSC/0.1% SDS; for the A_{1896}HBV probe: 5× SSC/0.1% SDS. The detection was carried out with alkaline Phosphatase conjugated anti-digoxigenin antibody (Boehringer Mannheim) and Lumi-Phos 530 (Boehringer Mannheim) as a substrate. After incubation at 37°C for 15 min, the membranes were exposed to an X-ray film (MP film, Amersham, Buck., UK) for 30 min and 2 hr.

For quantitation, the membrane was cut in small squares containing the dot using the pencil mark as guide, placed in Pony Vial H/I tube (Packard, Berks, UK) and 150 μl of 1:2 Lumi-Phos 530 diluted in alkaline phosphatase buffer (0.1 M Tris, 0.1 M NaCl, 0.05 M $MgCl_2$, pH 9.5), was pipetted on top of the dot, as described previously [Selph et al., 1993]. After 15 min at 37°C, the light emission (luminescence) was recorded using a liquid scintillation counter (Packard Tri-Carb 4640; window setting: 0–200 kEV) set on 30 sec/sample on continuous count mode. The values in the individual samples were read against the corresponding standard curve for the wild-type or precore mutant amplicons, respectively. In patients with mixed infection, the proportion between the two strains was presented as a percentage.

Automated sequencing of random samples with P3, as a sequencing primer, was used to confirm the results of the oligohybridisation assay and to exclude the presence of other precore stop codon mutants.

Routine Assays for Hepatitis Markers

HBsAg, HBeAg, anti-HBe, anti-hepatitis delta virus (HDV), anti-HCV, and anti-HIV were tested using commercially available assays (Abbott Diagnostics, Maidenhead, UK, Sorin Biomedica, Saluggia, VC, Italy and UBI HCV EIA, United Biochemical, Lake Success, NY). Serum HBV DNA was measured using a quantitative liquid-phase hybridisation assay (Genostics, Abbott Laboratories, Maidenhead, UK).

RESULTS

Of the two patients who did not receive HBIg after liver transplantation, one remained always positive for HBsAg in the serum, whereas the other was initially HBsAg negative and became seropositive for HBsAg

TABLE II. Hepatitis B Markers and Liver Histology After Liver Transplantation

	Pre-OLT		Reinfection				End follow up			
Pt	HBeAg	e-minus Mut %	Months post-OLT	HBeAg	HBV DNA (pg/ml)	e-minus Mut %	HBeAg	HBV DNA (pg/ml)	e-minus Mut %	Histology
JC	+	0		HBsAg		40*	–	2.9	57	Ci
KG	+	0	6	+	86	50	+	75	50	CAH
PJ	+	0	3.5	+	ND	ND	+	61	0	Ci
WM	+	0		HBsAg		0*	+	812	46	CPH
AS	–	85	6.3	–	33	85	–	11	66	CAH
AB	–	55	1.7	–	40	62	–	0	50	CAH
GC	–	80	1.5	–	ND	43	–	179.5	60	FHF
KK	–	40	9.4	+	16.5	40	+	22	46	mCAH
DL	–	0	17.2	–	2	33	–	0	33	mCAH
JP	–	50	1.2	+	1282	92	+	974	50	FCH
FS	–	0	1.9	–	ND	0	+	3	50	FHF
RS	–	80		HBsAg		40*	+	130	40	Ci
AQ	–	100	2.83	–	196	100	–	0	70	Ci
CK	–	100	1.2	–	ND	100	–	1147	100	mCAH

OLT, orthotopic liver transplantation; Pt, patient; HBeAg, hepatitis B e antigen; Mut, mutation; HBV, hepatitis B virus; HBsAg, persistent hepatitis B surface antigen positivity after OLT; ND, not determined; MHC, minimal hepatic changes; mCAH, mild chronic active hepatitis; CAH, chronic active hepatitis; Ci, cirrhosis; FCH, fibrosing cholestatic hepatitis; FHF, fulminant hepatic failure.
*Early post-transplant.

1.7 months after OLT. Among the 12 patients who received HBIg immunoprophylaxis, 2 patients (HBeAg positive with high serum HBV-DNA level) remained HBsAg positive continuously after transplantation. HBV recurrence was diagnosed between 1.2 and 9.4 months in 9 patients; in one case, HBV recurred at 17.2 months after OLT. In 4 of the 10 patients who received HBIg, HBV-DNA was detected in serum by nested PCR, whereas anti-HBs levels were still high 2–6 months before HBsAg became detectable in serum.

At the time of reinfection, the four patients who were HBeAg positive before the operation were also positive for HBeAg in serum. One of these patients seroconverted to anti-HBe 52.7 months post-OLT. Amongst the 10 patients who were anti-HBe positive before transplantation, HBeAg reappeared in serum at the time of reinfection in 4 and they remained HBeAg seropositive until the end of follow up (Table II). The other 6 patients were always seropositive for anti-HBe.

Changes in HBV Population

Before transplantation, an HBV population with a wild-type precore region was present in all 4 HBeAg-positive patients and in 2 of 10 anti-HBe-positive patients. Of the other 8 patients, 6 showed the presence of a mixed population of the two strains and 2 patients were infected with the pre-core mutant A_{1896}HBV alone. HBV recurrence was caused by the same viral population, with respect to the proportion of wild-type and precore mutant, which was present pre-OLT in 11 of the 14 patients studied: pure wild type in 4, mixed in 5, or pure A_{1896}HBV precore mutant in 2. In the remaining 3 cases, wild-type HBV population alone was present in 2, and mixed viral population in 1 before transplantation, whereas at the time of reinfection, these 3 patients showed a mixed population with predominance of the precore mutant.

During the posttransplant follow up, A_{1896}HBV mutant appeared and gradually accumulated in five of the six patients who had the wild-type pre-core HBV strain at the time of OLT (Fig. 1). In one case, who seroconverted from HBeAg to anti-HBe 52.7 months after OLT, A_{1896}HBV mutant became the predominant population (Fig. 2). The relative amounts of the two viral strains showed fluctuations during the entire follow-up period in five of six patients who had mixed viral population before OLT (Figs. 3 and 4). In the two patients in whom only a pre-core mutant A_{1896}HBV population was detectable before OLT, the wild-type HBV emerged in one, as part of a mixed population, 10 months after OLT. In the other patient, only the precore mutant population was found during the entire follow up.

Relationship to Liver Damage

Changes in the composition of HBV population were observed in the three patients who developed acute liver failure after HBV recurrence. The first patient had mixed HBV population with predominance of the precore mutant strain before OLT (Fig. 5). One month after reinfection, the proportion of wild-type HBV strain peaked (83%) in parallel with increasing viral replication (HBV DNA = 1,212 pg/ml). The patient died 25 days later and at that time, the proportion of the wild-type HBV had decreased to 40%. The second patient was infected with wild-type HBV before the operation and at the time of HBV recurrence. Two months posttransplant, mixed viral population was detected (50%), which persisted at the time when the patient developed FH and died. The third patient had a mixed viral population before transplantation. HBV DNA was detected in serum by PCR 15 days before

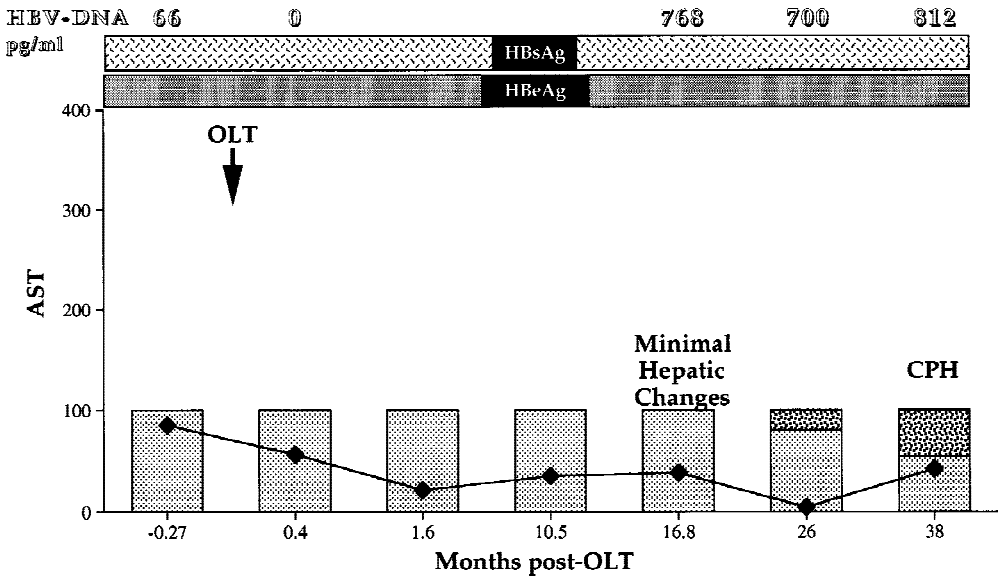

Fig. 1. Longitudinal analysis of the wild-type precore hepatitis B virus (HBV) (■) and precore A_{1896}HBV mutant (▩) in hepatitis B e antigen (HBeAg)-positive liver transplant recipient (WM). HBsAg, hepatitis B surface antigen; OLT, orthotopic liver transplantation; AST, aspartate transaminase; CPH, chronic persistent hepatitis.

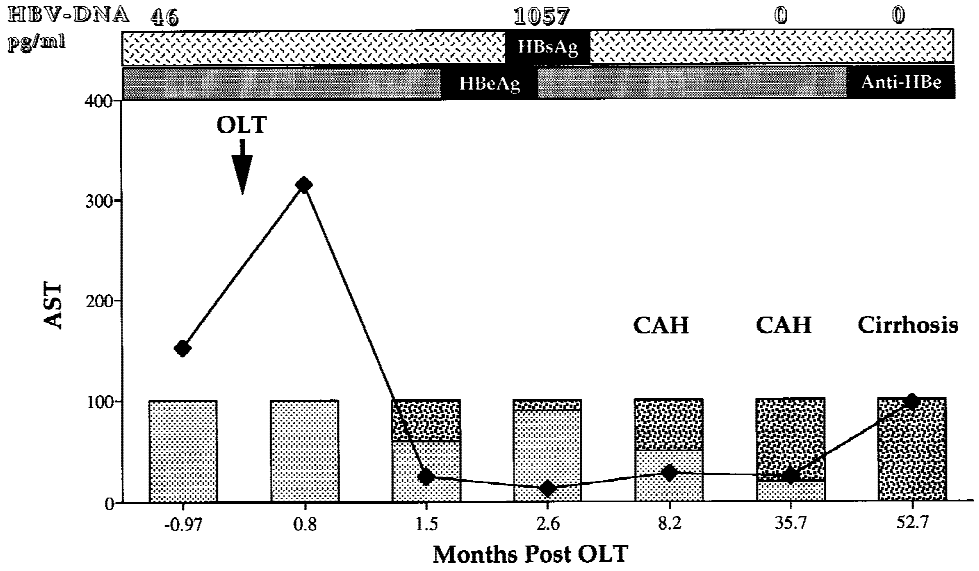

Fig. 2. Accumulation of precore A_{1896}hepatitis B virus (HBV) mutant (▩) in a liver transplant recipient (JC) who seroconverted from hepatitis B e antigen (HBeAg) to anti-HBe. HBsAg, hepatitis B surface antigen; OLT, orthotopic liver transplantation; AST, aspartate transaminase; CAH, chronic active hepatitis.

clinical reinfection. HBV recurrence, 1.2 months after OLT, was caused by mixed HBV population with high level of viral replication (HBV-DNA = 1,282 pg/ml), which persisted when the patient developed FCH and died 3 month post-OLT.

The relationship between the composition of HBV population and liver graft damage was analysed in 11 patients with chronic HBV infection after transplantation. Liver graft histology at 1 year post-OLT showed moderate to severe chronic hepatitis in 7, mild hepatitis in 2, and minimal histological changes in 2 patients.

In six patients with a longer follow-up period (median 50.8 months, range 38.3–71.1 months), cirrhosis developed more than 2 years after reinfection in four, one patient had severe chronic hepatitis, and one had mild hepatitis. There was no relationship between the liver histology and the type of viral population at reinfection, or at the end of the follow-up period.

DISCUSSION

We investigated the evolution of the wild-type and precore mutant A_{1896}HBV in liver transplant recipi-

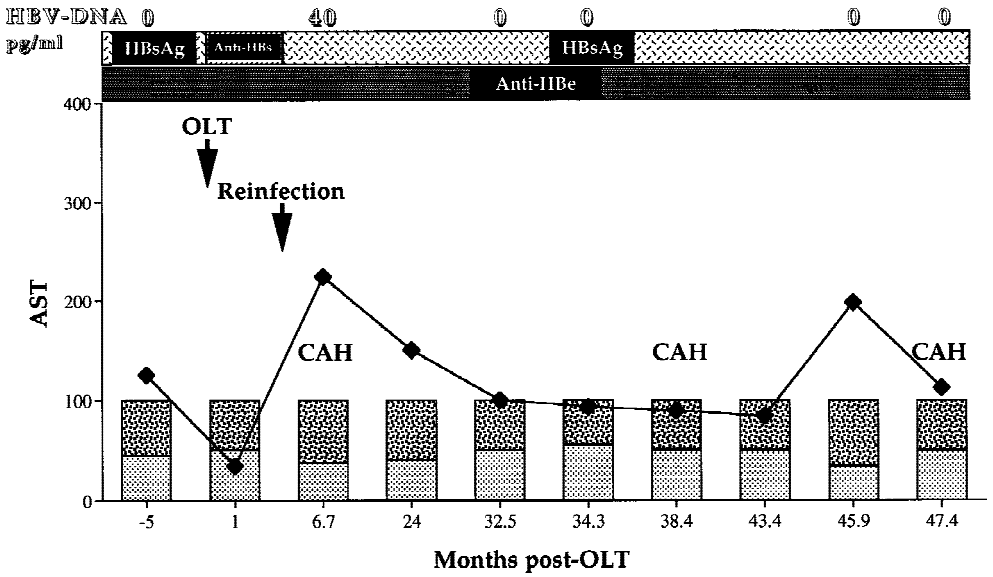

Fig. 3. Fluctuation of the wild-type precore hepatitis B virus (HBV) (■) and the precore A_{1896}HBV mutant (▩) in a liver transplant recipient (AB) persistently infected with a mixed virus population. HBsAg, hepatitis B surface antigen; OLT, orthotopic liver transplantation; AST, aspartate transaminase; CAH, chronic active hepatitis.

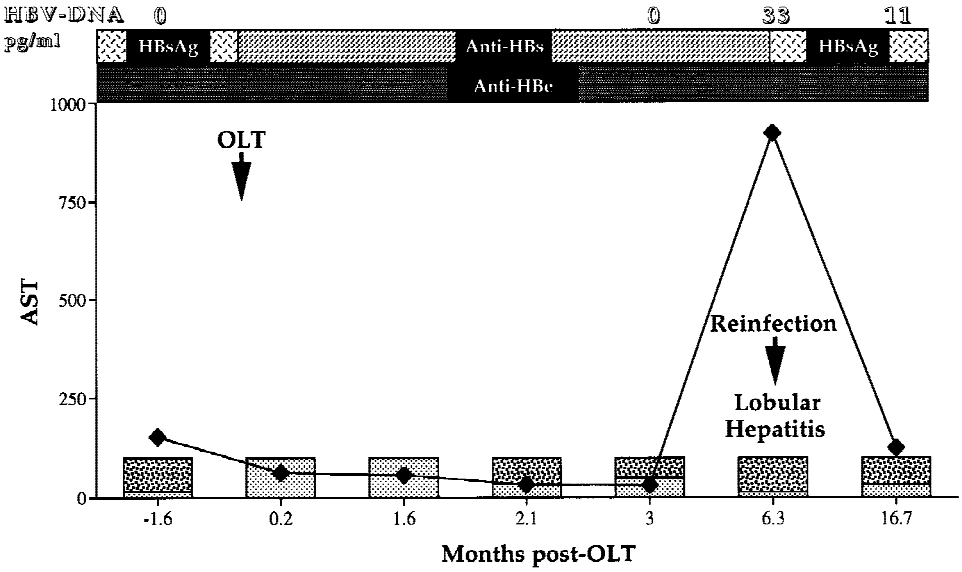

Fig. 4. Changes in the proportion of wild-type precore hepatitis B virus (HBV) (■) and precore A_{1896}HBV mutant (▩) in a liver transplant recipient (AS) who had HBV-DNA detectable by polymerase chain reaction before clinical reinfection. HBsAg, hepatitis B surface antigen; OLT, orthotopic liver transplantation; AST, aspartate transaminase.

ents with HBV recurrence and their impact on liver graft pathology. By quantitation of these two HBV types before and in serial samples after liver transplantation, it was demonstrated that the HBV strains with A_{1896}HBV stop codon gradually accumulated during the follow-up of liver transplant patients with hepatitis B, both in cases originally harbouring the wild type alone or in those infected with a mixture of the wild-type and A_{1896}HBV mutant.

Several case reports found the presence of A_{1896}HBV precore mutant in liver, kidney, or bone marrow transplant recipients with hepatitis B who developed fibrosing cholestatic hepatitis [Angus et al., 1993; Fang et al., 1993; Chen et al., 1994; McIvor et al., 1994; Booth et al., 1995]. However, other studies of patients with FCH found no particular association with the precore mutant HBV [Ackrill et al., 1992; Ghany et al., 1995; Lok et al., 1995]. In a series of liver transplant patients from Australia, it was suggested that assessment of the pretransplant HBV status should include testing for precore mutant strains, as liver transplant recipients with recurrent infection with A_{1896} HBV have a greater

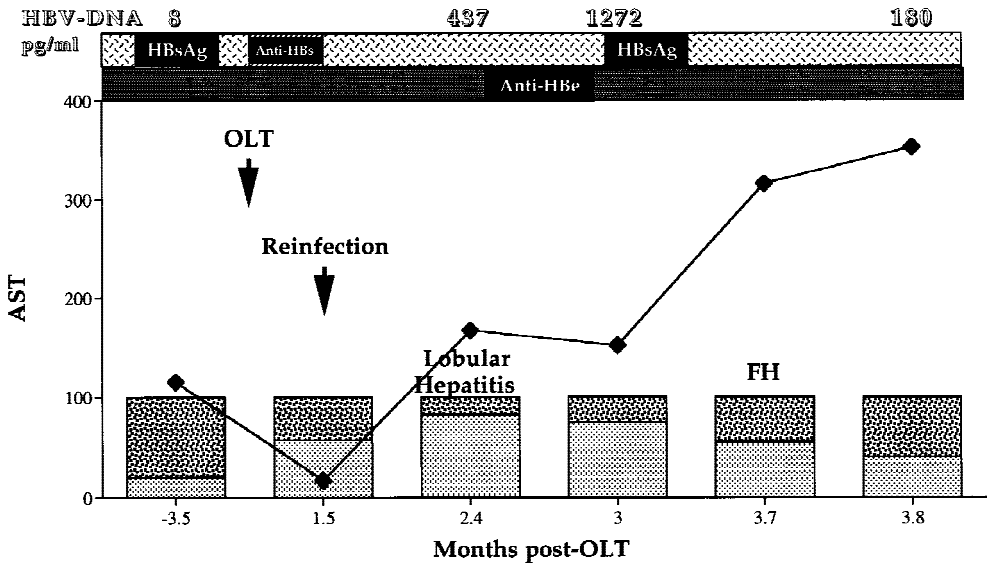

Fig. 5. Longitudinal analysis of wild-type precore hepatitis B virus (HBV) (■) and precore A_{1896}HBV mutant (▩) in a liver transplant recipient (GC) who died of fulminant hepatitis B early after transplantation. HBsAg, hepatitis B surface antigen; OLT, orthotopic liver transplantation; AST, aspartate transaminase; FH, fulminant hepatitis.

risk for graft loss and death than patients reinfected with HBV wild type [Angus et al., 1995]. Factors other than the particular HBV strain may have played a significant role in determining the severity of graft injury in these patients, as we have shown previously that the severity of hepatitis B in cases infected with the precore mutant HBV correlates with the level of HBV replication [Naoumov et al., 1992]. In a large group of liver transplant recipients, Naumann et al. [1997] showed that pretransplant infection with precore mutant HBV does not have a negative impact on the rate of HBV recurrence, HBV-related graft failure, patient survival, or the severity of hepatitis B in the graft. The results of the present study support this observation and in our series there was no relationship between liver graft histology and the type of viral population. Furthermore, the design of the present study with serial testing of the viral population after HBV recurrence revealed a gradual accumulation of the precore mutant HBV during posttransplant follow up. The emergence of A_{1896} precore stop codon is believed to result from the host's immune pressure against the virus, either during the natural evolution of chronic HBV infection or when interferon is given [Takeda et al., 1990; Marinos et al., 1996]. Our data indicate that immune selection of A_{1896}HBV continues to occur in liver transplant patients despite immunosuppression with cyclosporine and azathioprine. This finding is supported by immunological studies showing that liver transplant recipients with HBV recurrence are able to mount HBcAg-specific T-helper cell proliferation and cytokine release [Marinos et al., 1995] and the ability of these patients to respond to vaccination with HBsAg [Duvoux et al., 1995]. In one of the liver transplant patients studied here, this response was a long-standing process and along with the accumulation of A_{1896}HBV, he developed spontaneous seroconversion to anti-HBe, similar to the seroconversion in nontransplant patients with chronic hepatitis B. The longitudinal analysis of patients with acute liver failure due to HBV recurrence reveals rapid evolution of HBV population in the two patients who developed fulminant hepatitis B in the graft. The immune selection of the precore mutant was condensed within a much shorter period of time, with changing prevalence from wild-type predominance at the time of reinfection to precore mutant HBV predominance at the time of death.

HBV recurrence in the new liver is usually due to the same viral population that was present before OLT [Laskus et al., 1994; Protzer et al., 1996]. Experimental mixed infections of ducks or cell lines comparing the replication of these two strains have shown that the wild-type HBV can replicate more efficiently in the absence of selection pressure [Tong et al., 1991; Chuang et al., 1994]. In patients harbouring mixed populations of wild-type and A_{1896}HBV before the operation, recurrence after transplantation allows comparisons of the fitness of these two strains in a human system. The present study indicated that wild type was more readily detectable in the first months after transplantation, which may be due also to the triple immunosuppression including steroids at this stage, whereas during the long-term follow up when immunosuppression is much reduced, there was a tendency for the precore mutant to accumulate. In some liver transplant patients, it has been shown that the wild type may become predominant, which is probably due to the higher immunosuppression used, including anti-lymphocytic antiserum and continuous corticosteroid treatment [Protzer et al., 1996]. As exceptions, we observed two patients who showed only the wild-type HBV in the pretransplant sample, but who had a significant pro-

portion (30% and 50%) of A_{1896}HBV strains at the first sample of HBV recurrence after OLT. It is possible that such different HBV population is originating from an extrahepatic reservoir harbouring this HBV mutant, for example peripheral blood mononuclear cells as shown previously in individual cases [Brind et al., 1997].

The present study supports the notion that severe chronic hepatitis B after liver transplantation or HBV related acute graft failure is not necessarily related to the precore mutant HBV strain. Further studies are needed to evaluate the prophylaxis of HBV recurrence with HBIg with or without antiviral agents as the most effective approach to improve survival in patients transplanted for HBsAg-positive cirrhosis.

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